A rapid screening test for reduced fibrinolytic activity of plasma: streptokinase activated lysis time

E. GIDRON, R. MARGALIT, AND Y. SHALITIN

From the Clinical Research Laboratory, Department of Medicine B, Rothschild University Hospital and Department of Biology, Technion-Israel Institute of Technology, Haifa, Israel

SUMMARY A simple screening method for determining the fibrinolytic activity of plasma is described. A streptokinase activated system is used, which measures the result of the interplay of all components of the fibrinolytic system with the exception of activators, which are added in excess. Mean lysis time and standard deviation with this method is 6.9 ± 1.4 min. The sensitivity of the method in detecting minor delays in clot lysis time is demonstrated.

The methods available for the determination of reduced fibrinolytic activity of plasma are few and are not easily applied to individual cases owing to wide limits of normal. The dilute blood clot lysis time (Fearnley et al., 1957) has an arbitrary normal limit of 7 hours, but many normal clots lyse after 3-4 hours; for them 7 hours is an abnormal value. Similarly, the euglobulin lysis time (von Kaulla and Schultz, 1958) has a normal range of 2.5 to 4 hours, representing the lysis time of a clot with most inhibitors removed. In the fibrin plate method (Astrup and Mülertz, 1952) the area of lysis is measured, all the inherent inaccuracies of such measurements being increased by the small size of the area when lysis is delayed. Hickman's (1971) method using labelled fibrinogen is accurate but rather cumbersome.

The purpose of this paper is to describe a simple method for the determination of the fibrinolytic activity of plasma in an activated system. This method reflects the influence of all the components of the complex fibrinolytic system with the exception of activators, which are added in excess.

The method is based partly on the observations of Kontinenen (1965), who investigated the influence of various concentrations of streptokinase (SK) on plasma clot lysis time, and on the urokinase (UK) sensitivity test of McNicola et al. (1963).

Materials

1 Streptokinase (VaridaseR, Lederle): The stock solution containing 10 000 U of SK per ml of phosphate buffer was kept at −20°C and was stable for two weeks.

2 Thrombin (Sigma): The stock solution containing 50 U/ml of phosphate buffer was kept at −20°C in aliquots of 2 ml and was stable for two weeks.

3 Urokinase1: Aliquots of 2 ml containing 480 CTA units per ml were stored in the dark at 0°C.

4 Human Fibrinogen (Kabi): coagulability of 95%.

5 Plasminogen (Profibrinolysin, Sigma): Aliquots of 1 ml containing 5 U of plasminogen were stored at 0°C.

6 Phosphate buffer: pH 7.4, 0.15 ml.

Methods

1 Blood sampling: 4.5 ml of blood was drawn from fasting subjects between 0800 and 0900 hours and placed in tubes containing 0.5 ml of sodium citrate (3.8%).

2 Fibrinogen was determined by the method of Godal (1961). The normal range with this method is 2.0-4.0 g/l.

3 Plasma streptokinase lysis time: 0.1 ml streptokinase in concentrations of 100, 150, 200, 500, 1000, and 2500 U/ml of plasma was added to test tubes containing 0.1 ml of plasma and 0.8 ml of phosphate buffer, and the mixture was clotted by an addition of 5 U of the stock solution of thrombin. The lysis time was defined as the time elapsing from the moment of adding thrombin until complete lysis of the clot.

4 Dilute blood clot lysis time was performed by the method of Fearnley et al. (1957). The end point was determined visually or photographically;

Received for publication 8 July 1977

1Kindly supplied by Dr Alan Johnson
the normal range in 35 controls was in our laboratory 3-5 hours.

5 Streptokinase activated lysis time (SALT): The blood samples were centrifuged at room temperature for 30 min at 3000 rpm, and the plasma was separated. 0-15 ml of a 100 U/ml solution of streptokinase was added to the test tubes containing 0-1 ml of plasma and 0-65 ml of phosphate buffer, resulting in a final concentration of 150 U SK per ml of plasma. The mixture was clotted by the addition of 0-1 ml of the stock solution of thrombin. The end point of lysis was determined visually or by a nephelometric method. The lysis time was defined as for plasma streptokinase time above.

6 Nephelometric method for recording lysis time: A Turner 111 Fluorimeter with a recorder was used to register the clotting and lysis of plasma by the SALT method. A 400 nm excitation filter with a 1% neutral density filter was used without an emission filter. The different light scattering properties of the plasma solution and the clot permitted the plotting of coagulation and lysis: the fluorimeter was calibrated with the plasma-SK mixture, and the return of the curve to the base line was the end point. There was close agreement between the visual and nephelometric SALT. Typical curves can be seen in Figure 5.

Results

LYSIS TIME WITH DIFFERENT CONCENTRATIONS OF SK

Figure 1 shows the lysis time of 18 random plasma samples from inpatients as a function of increasing concentration of SK. The optimal concentration, resulting in the shortest lysis time, was found to be 500 U/ml, both lower and higher concentrations prolonging the lysis time. This is in full agreement with the findings of Konttinen (1965). While there was a certain amount of scattering of lysis time with all concentrations of SK, we found that 150 U/ml of plasma gives an optimal range of results in normal individuals and a sufficient scattering of lysis time in patients, and adopted this concentration for the SALT.

INFLUENCE OF FIBRINOGEN AND PLASMINOGEN CONCENTRATIONS ON LYSIS TIMES

Human fibrinogen was added to plasma, resulting in concentrations of 3-7-16-5 g/l. To each test tube containing 0-1 ml of fibrinogen-enriched plasma 0-2-0-6 U of plasminogen was added and the test was performed as for SALT. Figure 2 shows that the lysis time is getting longer as the fibrinogen concentration rises, and is shortened by increasing the plasminogen contents.
The interrelations between fibrinogen and plasminogen levels and their influence on lysis time are shown in Fig. 2; the rise in plasminogen level observed by us (Gidron et al., 1977) after myocardial infarction may be part of a homoeostatic mechanism counteracting the rise in fibrinogen and inhibitor levels which precedes it. Similarly, the scattering of the lysis time in normals and random patients, seen in the SALT test, may be due to variations in the levels of the various components of the fibrinolytic system; it is unlikely that it is due to varying antistreptokinase levels because the use of higher concentrations of SK (2500 U/ml) resulted in similar scattering. Moreover, 150 U/ml of SK correspond to a dose of approximately 500,000 U in an adult, more than sufficient to neutralise any anti-SK levels likely to occur, except those after streptokinase treatment. We considered that the high concentrations of SK introduce an additional extraneous factor into the reaction and preferred the activation of the system by 150 U SK per ml of plasma. In cases showing marked delay in lysis a control test with 2500 U-SK/ml may be performed if there is reason to suspect a high anti-SK titre. The mean lysis time and standard deviation with this concentration is 6.7 ± 1.9 min.
A rapid screening test for reduced fibrinolytic activity of plasma: streptokinase activated lysis time

References


Fig. 5 Plasma lysis time on days 1, 4, 5, 8, and 11 after myocardial infarction (nephelometric recording).
A rapid screening test for reduced fibrinolytic activity of plasma: streptokinase activated lysis time.

E Gidron, R Margalit and Y Shalitin

J Clin Pathol 1978 31: 54-57
doi: 10.1136/jcp.31.1.54

Updated information and services can be found at:
http://jcp.bmj.com/content/31/1/54

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/